E-ISSN: 2347-5129
P-ISSN: 2394-0506
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.549
IJFAS 2019; 7(5): 478-481

www.fisheriesjournal.com
Received: 18-07-2019
Accepted: 22-08-2019

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Masculinine effects of *Nigella sativa* on Tilapia

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Abstract
This research was conducted in order to investigate the sex reversal effects of *Nigella sativa* seed extract on Tilapia. The *Nigella sativa* seeds aqueous extract were prepared according to standard method. *Tilapia zilli* broodstock were collected and allowed to bred, afterward the fry's were immediately collected with a scoop net and randomly assigned into concrete tank to four different treatment groups (0.0 or control, 0.05, 0.1 and 0.15 g/l) in three replicates. Sexes of the tilapia were determined according to the standard method of sex determination. The result shows that the percentage survival rate in controls was similar to those in the treated groups. The highest percentage (86.67%) of sex reversal was observed in the group treated with 0.15g/l *Nigella sativa* seed aqueous extract at 0.15g/l concentrations. while the group treated with 0.2g/l concentration had the least percentage of sex reversal (71.67). Qualitative phytochemicals analysis of *Nigella sativa* revealed the presence of alkaloids, flavonoids, steroid, tannins and saponins. The results indicates that, *Nigella sativa* seed extract could be used as an alternative method to produce all-male tilapia population in an environment-friendly manner through the use of this natural product.

Keywords: *Nigella sativa*, Sex reversal, phytochemicals, *Tilapia zilli*

Introduction
Tilapia, belong to the family Cichlidae is one of the important dietary protein sources in many countries of the world. The word tilapia is derived from the African native Bechuana word "thiape", meaning fish [1]. They are endemic to Africa and Middle East but have been taken to and stocked in waters of nearly every country of the world for aquaculture [2]. In developing countries more than 20 species of tilapia have been cultured [1].
Rapid growth rates, high tolerance to low water quality, efficient food conversion, resistance to disease, good consumer acceptance and ease of spawning made tilapia a suitable fish for culture [3]. The fish is reported to sexually mature at a small size of around 6 cm and a young age of around 3 months [2]. Sex invasion in tilapia fishes is a well documented fact, that is gonadal sex differentiation can be re-directed by many other factors, which includes environmental factors such as temperature and pH [4], and exogenous endocrine-active chemicals [5]. 17-αmethyltestosterone (MT) release and environmental concerns are major reason behind the search for alternative, environment-friendly chemicals. Within the contemporary atmosphere of increasing governmental regulation on the use of chemicals on food fish, continued dependency on steroid-induced monosexing places the culture of tilapias in a precarious position [3]. Therefore, safe alternative methods and chemicals are necessary in the production of monosex tilapia populations [6]. One of such methods is the use of phytochemicals. Phytochemicals are group of plant-derived chemicals that are commonly found in cereals, fruits, vegetables, and plant-based beverages like tea and wine [7]. These Phytochemicals have been reported to enhance the growth, feed consumption, antimicrobial, immunostimulation, and antistress properties of fish [7-10].
Nigella sativa commonly known as the fennel flower plant, has finely divided foliage and blue flowers, which grows to a maximum height of about 60 cm and it produce black seeds. The common and local names of the plant are black cumin and Habba-tu respectively. Extensively it is cultivated in India and Pakistan [10].
N. sativa seeds, as nutritional and medicinal plant, have traditionally been used for thousands of years as folk medicine and some of its active compounds were reported against many ailments. Different pharmacological effects such as gastric ulcer healing, anti-microbial effect, anti-cancer activity,

cardiovascular disorders, gastroprotective and antioxidant activity, immunomodulatory, anti-inflammatory and antitumor effects, antitussive effect, anti-anxiety effect, anti-asthmatic effect, anti-inflammatory effects in pancreatic cancer cells, anti-helicobacter activity, tumor growth suppression, anti-viral activity against cytomegalovirus, hepatoprotective activity have been reported for this medicinal plant [11].

Tilapia species has high breeding rate and usually produce many offspring within a short time. This often leads to unconducive environment, culminating in stunted growth and economic loss. Synthetic hormones are used to solve this problem. However, there is a great debate concerning the heath effect of using synthetic hormone technique on fish consumers and fish hatchery waste water, which may be drained into surface water. 17-αmethyltestosterone (MT) which is used for sex reversal of tilapia has carcinogenic effects [12]. The increased use of synthetic steroid hormones to produce monosex populations of tilapia for intensive productive systems may lead to environmental and public health concerns. MT is also known to be associated with gonad malformation problems when administered at high doses or over a prolonged time. MT induces gonadal intersexuality and paradoxical feminization [13]. Chronic exposure of consumers to synthetic steroids may cause adverse health effects and its use in fish culture is associated with potential release to the environment and contamination of the biota [4]. Thus the main aim of this study is to produce all male *Tilapia zillii* using *Nigella sativa* seed extract.

Materials and Methods

Experimental site

This research was conducted at the fisheries and Hydrobiology Research unit, Department of Biological Sciences, Kebbi State University of Science and Technology, Aliero. The town is located in the northwestern Nigeria on latitude 113 ^{0}S. 12.44 ^{0}N latitude 36 ^{0}W. 42 ^{0}E [14].

Acquisition of tilapia hatchlings

Forty brood stocks of Tilapia were collected from Danbaba`s Farm, Jega. The fishes were transported in an open 25L white plastic container to the research. The brood stocks were released by placing the container inside the concrete tank containing water and fish were allowed to swim out. Broodstock were allowed to breed, afterward the Frys were immediately collected with a scoop net and transferred into experimental tank.

Acquisition, identification and preparations of plant extracts

The dried seeds of *N. sativa* seeds were bought at Birnin Kebbi central market, and authenticated at the herbarium section of the Biological Sciences Department of the Kebbi State University of Science and Technology Aliero. The plant seed were grinding with a blender, 100g of ground seed were soaked in 500 ml aqueous solution for 24 hours with constant shaking at intervals as described by Musa [15]. It was filtered using Watman filter paper, the filtrate were concentrated by drying it in the oven at a temperature of 40°C for 8 hours. The concentrated extract were stored in clean bottle, labeled and then preserved in the refrigerator until when needed.

Experimental Design

720 of 5days old mixed sex of Tilapia fry were randomly assigned into 12 experimental tank to four different treatment groups (0.0 or control, 0.05, 0.1 and 0.15 g/l), i.e. 60 fish sample per treatment in 3 replicates. The experiment lasted for 60 days during which fish were immersed into the plant extracts for a period of 2hours once in a week. Immersion was done by transferring the fish in to plastic bows (120L capacity) containing varying concentrations (0.0, 0.1, 0.15 and 0.02g/l) of the plant extract. The experiment was conducted in the morning period and the tanks were continuously aerated and maintained in heated (T = 27 ^{0}C) static systems and the fish was kept under similar photoperiod to ensure there is no disparity Water in all concrete tanks were replaced weekly. The fish were fed with the same quantity of commercially imported diet containing 35% crude protein at a rate of 20% body weight/day.

Phytochemical screening

The phytochemical analysis of plant extract were carried out a ccording to the Standard methods of analysis [16].

Determination of some physicochemical parameters of culture water

The dissolved oxygen, temperature, total dissolved solid and pH of the water in tanks were determined bi-weekly as describe by Obaroh and Nzeh [17].

Sex determination

At the end of the experiment, sex of the tilapia fish were determined anatomically by examining their internal and external reproductive organ. A total of ten fish in each treatment were randomly collected in all the replicates and sex were confirmed internally by dissection through examination of their gonads as described by Bamisaye [18].

The external sexing was done using the method described by Guerrero and Shelton [19] through examining the genital papilla which is located immediately behind the anus. In males *Tilapia* spp the genital papilla has only urinary pore of the ureter, through which both milt and urine pass. In females the genital papilla has separate oviduct and urinary pore [19].

Data analysis

Data collected in this study expressed in Percentage

Results and Discussions

Masculinine of *T. zilli* per concentration of *Nigella sativa* seed extract

All the treatment categories showed higher percentage of males compared to the control. The highest percentage (86.67%) of sex reversal of tilapia fry was observed in the ponds treated with *Nigella sativa* seed aqueous extract at 0.15g/l concentrations. This was followed by the ponds treated with 0.1g/l concentration with a percentage sex reversal of 86.67 while, the ponds treated with 0.2g/l concentration had a least percentage of sex reversal of 71.67 (Table 1).

Table 1: Masculinine of *T. zilli* per concentration of *Nigella sativa* seed extract

Treatment	% No. of male	% No. of female
A (control/ 0.00)	28 (46.67)	32 (53.33)
B(0.1g/l)	49 (81.67)	7 (11.67)
C(0.15g/l)	52 (86.67)	6 (10)
D(0.2g/l)	43 (71.67)	12 (20)

Survival rate after the treatment

The highest survival percentage (96.67%) was observed in treatment with *Nigella sativa* seed aqueous extract at the concentration of 0.15 g/l while the lowest survival percentage was observed in treatment with *Nigella sativa* seed extract at the concentration of 0.1g/l (93.33%) (Table 2)

Table 2: Survival rate after the treatment

Treatment	Initial No. of fishes	Mortality	Percentage Survival (%)
A (control/ 0.00)	60	0	60 (97.77)
B(0.1g/l)	60	4	56 (93.33)
C(0.15g/l)	60	2	58 (96.67)
D(0.2g/l)	60	5	55 (94.67)

Qualitative phytochemical screening of *Nigella sativa* seed extract

Qualitative analysis for phytochemicals revealed the sparingly presence of Alkaloids, steroid and Saponins. It also shows the highly presence of Tannins and Flavonoids in *Nigella sativa* seed extract, while Glycoside are not present in the extract (Table 3).

Table 3: Result of qualitative phytochemical screening of *Nigella sativa* seed extract

Phytochemicals	Screening test
Alkaloids	+
Flavonoids	++
Glycoside	-
Steroids	+
Tannins	++
Saponins	+

+ = Sparingly present, + + = highly present, - = Not present

Physicochemical parameters of water used for culture

The result shows that the temperature of all treated group ranged from 27.67±0.20 to 28.94±0.14ºC. The dissolved oxygen (DO) in all treatment ponds ranged from 3.71+0.25to 3.89+0.16mg/l. The pH treatment ponds ranged from 6.98+0.01 to 7.07+0.06. While Total dissolved solid (TDS) of all treatment ponds ranged between 157.18+0.61 to 210.42+1.62pp/m. Furthermore, the result on temperature and TDS shows that there is significant differences between the control and other treatment groups. While the result of pH and DO show no significant Differences among the groups (Table 4).

Table 4: Physicochemical parameters of pond water used for culture

Treatment/Parameters	Temperature (^{0}C)	pH	TDS (pp/m)	DO (mg/l)
A (control)	27.67+0.20^a	7.05+0.13^a	190.49+8.71^b	3.75+0.26^a
B(0.1g/l)	28.79+0.20^b	6.98+0.01^a	210.42+1.62^c	3.71+0.25^a
C(0.15g/l)	28.94+0.14^b	7.07+0.06^a	186.35+1.05^b	3.80+0.15^a
D(0.2g/l)	28.70+0.34^b	7.04+0.05^a	157.18+0.61^a	3.89+0.16^a

Values are expressed as mean ± SD.

Mean with the same superscript letter in the same column is not significantly different (p>0.05) and vice versa.

TDS= Total dissolved solid; DO= Dissolve Oxygen

Discussion

The results shows highest percentage of males in all treated groups except the control (Table 1), this infers that *Nigella sativa* posses androgenic property which has been found to be effective in *T. zillii.* This findings is in agreement with the study done by Phelps and Popma [20] who found higher percentage of male Tilapia when treated with plant extract. The masculine effect might be due to the presence of flavonoids, saponins and steroids in *N. sativa*, which are natural compounds characterized by androgenic activity[10, 21]. Moreover, *Nigella sativa* seed has been reported to be used in traditional medicine to treat sexual asthenia and infertility in man [21].

However, the highest percentage of males produced by immersion in *Nigella sativa* seed extract is 86.67%, which is below the ideal requirement of 100% male population. Thus, further studies would be required to establish an ideal treatment regime for production of all-male tilapia population using *Nigella sativa* seed extract and to provide conclusive evidence regarding its efficacy to be used as a sex-reversal agent in tilapia culture. The failure to obtained 100% sex reversal using animal testes might be a result of the method of preparation of the extract (aqueous). As a result, it may have been possible that solvent used in the preparation of extract contribute in achieving 100% sex reversal in Tilapia fishes. This is because the ethanolic and methanolic extracs had been reported by Phelps and Popma [21] to induce 100% sex reversal in Tilapia fishes. Survival percentage in controls was similar to those observed in the *Nigella sativa* seed extract treated groups, where no significant dose-related inter-group differences were noted (Table 2). The result indicates that immersion treatment with *Nigella sativa* seed aqueous extract has no adverse effects on the survival of fishes.

Conclusion

This study indicated that immersion treatment with *Nigella sativa* seed aqueous extract has no adverse effects on fish survival. The phytoconstituents might render the androgenic activity of the extract. The results revealed that *Nigella sativa* seed extract might be used as an alternative method to produce all-male tilapia population in an environment-friendly manner using a natural product.

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